

Atty. Dkt. No. 038602/1180



Gregory PLOWMAN, *et al.*
Serial No. 09/866,987

CONCLUSION

If the Examiner has any questions concerning this application, he or she is requested to contact the undersigned.

Respectfully submitted,

By

A handwritten signature in black ink, appearing to read "Beth A. Burrous", written over a horizontal line.

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Date October 3, 2001

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.



VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 124, first full paragraph:

“cDNA libraries” derived from over 450 tissue or cell line sources were immobilized onto nylon membranes and probed with ³²P-labeled cDNA fragments derived from the gene(s) of interest. To make the cDNA, total RNA or mRNA was used as template in a reverse transcription reaction to generate single-stranded cDNAs (ss cDNA) that were tagged with specific sequences at each end. An oligo dT primer containing a specific sequence (CDS: AAGCAGTGGTAACAACGCAGAGTACT₃₀ VN (V=A,G,C N=A,G,C,T)) (SEQ ID NO: 11) anneals at the polyA track at the 3’ end of the mRNA and the reverse transcriptase (MMLV RnaseH⁺) transcribes the antisense strand until it reaches the end of the RNA strand when it adds additional C residues. If a primer (SMII: AAGCAGTGGTAACAACGCAGAGTACGCGGG (SEQ ID NO: 12) or ML2G: AAGTGGCAACAGAGATAACGCGTACGCGGG+ (SEQ ID NO: 13)) ending with 3 Gs is added, it anneals to the added Cs and the MMLV recognizes the rest of the primer sequence as template and continues transcription. As a result, the synthesized cDNAs contain specific sequence tags at both the 5’ and the 3’ end. When the 5’ and the 3’ ends are tagged with the same sequence (CDS and SMII) it is referred to as “symmetric”. When the 5’ end is tagged with a different sequence than the 3’ end (CDS and ML2G) is referred to as “asymmetric”. A double-stranded “cDNA library “ is then generated by PCR amplification using the 3’ PCR and ML2 primers (3’ PCR: AAGCAGTGGTAACAACGCAGAGT (Portion of SEQ ID NO: 11) and ML2: AAGTGGCAACAGAGATAACGCGT+ (Portion of SEQ ID NO: 13)) that anneal to the added sequence tags.

On page 125, first full paragraph:

In order to prepare a cDNA fragment for production of a ³²P labeled probe for SGP061, two oligonucleotides, 5’-TTGCGGAGCTTGACGCGC-3’ (SEQ ID NO: 14) and 5’-TCCCATCCTTTGTTGCCCG-3’ (SEQ ID NO: 15), were used to amplify a 430 basepair fragment by PCR. The fragment was purified by separation on an agarose gel and the

basepair fragment by PCR. The fragment was purified by separation on an agarose gel and the sequence was verified by using the same oligonucleotides as primers for the sequencing reaction. The PCR product was detectable in a range of tissue sources including prostate, placenta, salivary gland, skeletal muscle, spinal cord as well as many tumor cell lines. This cDNA fragment was then used to determine the expression of SGP061 on tissue arrays as described above. Initial comparison of normal tissue expression levels with tumor cell line expression levels revealed that SGP061 was elevated in a number of tumor cell lines including those derived from breast, colon, leukemia, lung, melanoma, glioblastoma, ovarian and renal tissue sources.

On page 137, last full paragraph:

Composition of Buffer

10x PanMix

5% Triton X-100

10% non-fat dry milk (Carnation)

10 mM EGTA

250 mM NaF

250 µg/mL Heparin (sigma)

250 µg/mL sheared, boiled salmon sperm DNA (sigma)

0.05% Na azide

Prepared in PBS

Wash Buffer

PBS supplemented with:

0.5% NP-40

25 µl g/mL heparin

PCR reaction mix

1.0 mL 10x PCR buffer (Perkin-Elmer, with 15 mM Mg)

0.2 mL each dNTPs (10 mM stock)

0.1 mL T7UP primer (15 pmol/µL) GGAGCTGTCGTATTCCAGTC (SEQ ID NO: 16)

0.1 mL T7DN primer (15 pmol/µL) AACCCCTCAAGACCCGTTTAG (SEQ ID NO: 17)

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0.2 mL 25 mM MgCl_2 or MgSO_4 to compensate for EDTA

Q.S. to 10 mL with distilled water

Add 1 unit of Taq polymerase per 50 μL reaction

LIBRARY: T7 Select1-H441